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In vitro metabolism of gestodene in target organs: formation of A-ring reduced derivatives with oestrogenic activity

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Abstract

Gestodene (13β-ethyl-17α-ethynyl-17β-hydroxy-4,5-gonadien-3-one), the most potent progestin ever synthesized, stimulates breast cancer cell growth through an oestrogen receptor-mediated mechanism, and its use in hormonal contraception has been associated with side effects attributable to oestrogenic actions. These observations have remained controversial, since gestodene does not bind to the oestrogen receptor or exert oestrogen-like activities. Recently, we have demonstrated that non-phenolic gestodene derivatives interact with oestrogen receptors and induce oestrogenic effects in cell expression systems. To assess whether gestodene is biotransformed to metabolites with intrinsic oestrogenic potency, [^{3}H]- and [^{14}C]-labelled gestodene were incubated in vitro with rat anterior pituitary, hypothalamus and ventral prostate homogenates under different experimental conditions. The most remarkable finding was the isolation and identification of 3β,5α-tetrahydrogestodene and 3α,5α-tetrahydrogestodene as metabolic conversion products of gestodene, presumably with 5α-dihydrogestodene as intermediate. The overall results seem to indicate that the weak oestrogenic effects attributable to gestodene could be mediated by its tetrahydro metabolites. © 2001 Published by Elsevier Science B.V.

Keywords: Gestodene metabolism; Synthetic progestin; Oestrogenic effect; Gestodene 5α-reduction

1. Introduction

Evidence has accumulated over the last years indicating that contraceptive synthetic progestins may exert a variety of hormonal agonistic, synergistic and even antagonistic effects, other than their progestational activities (Mowszowicz et al., 1974; Vilchis et al., 1986; Castro et al., 1995). A number of experimental studies have demonstrated that these hormone-like effects of synthetic progestins are mediated either by their interaction with the inappropriate steroid receptors (Bardin, 1983; Pérez-Palacios et al., 1983; Lemus et al., 1992) or by their metabolic conversion products (Chávez et al., 1985; Moralí et al., 1990; Pasapera et al., 1995). Recent studies have suggested that gestodene, a potent synthetic progestin widely used in the so-called third-generation contraceptive pills, may induce oestrogen-like effects apparently mediated by the oestrogen receptor (Van der Burg et al., 1992; Catherino et al., 1993; Kalkhoven et al., 1994; Schoonen et al., 1995). Furthermore, the use of gestodene has been associated with a relative low increase in venous thromboembolic disease (Jick et al., 1995; World Health Organization, 1995a,b; Lewis et al., 1996). These unexpected effects of the gestodene molecule prompted us to investigate whether this synthetic progestin exerts oestrogenic effects through some of its metabolites, particularly since gestodene is not recognised by the oestrogen receptor (Düsterberg et al., 1987). Further interest in conducting this study derived from a recent report from our laboratories showing that two gestodene derivatives, the 3β5α-tetrahydrogestodene and to a lesser extent its 3α isomeric alcohol, specifically bind, with a relative low binding affinity, to the oestrogen receptor and activate the human oestrogen receptor-α-mediated transcription of oestrogen-

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dependent genes in yeast and HeLa cells expression systems. Thus, the slight oestrogen-like effects attributable to gestodene may be mediated by two of its A-ring reduced metabolites (Lemus et al., 2000).

To assess the metabolic fate of gestodene at the target organ level, homogenates of female rat anterior pituitaries and hypothalami and male rat ventral prostates were incubated in vitro with [^{3}H]- and [^{14}C]-labelled gestodene under different incubation conditions in the absence or presence of NADPH. The radiochemical purity of isolated gestodene metabolites was established by a reverse isotope dilution technique. Incubations using [^{3}H]-labelled testosterone were used as the positive controls, while tissue-free incubations served as the negative controls.

2. Materials and methods

2.1. Steroids and chemicals

[9,11-^{3}H]-gestodene ([^{3}H]-gestodene) specific activity 30.54 Ci/mmol, [^{14}C-ethynyl]-gestodene ([^{14}C]-gestodene) specific activity 43.75 mCi/mmol and authentic radioinert GSD were kindly provided by Schering (Berlin, Germany). [1,2,6,7-^{3}H]-testosterone ([^{3}H]-testosterone) specific activity 85 Ci/mmol was purchased from NEN® Research Products (Boston, MA, USA), 5α-dihydrogestodene (5α gestodene) was synthesised by lithium-ammonia reduction of gestodene (Lemus et al., 2000), the 3α,5α gestodene tetrahydro derivative was prepared by reduction of 5α gestodene with L-Selectride under anhydrous conditions (Lemus et al., 2000) and the 3β,5α gestodene tetrahydro derivative was synthesised by sodium borohydride reduction of 5α gestodene (Lemus et al., 2000). The chemical purity of gestodene and its derivatives were assessed by their melting points, HPLC behaviour, infrared absorption, and [^{13}C]- and [^{1}H]-nuclear magnetic resonance. The physical and spectroscopic constants of the A-ring reduced gestodene derivatives have been previously reported (Lemus et al., 2000). Non-radioactive steroids and NADPH were supplied by Sigma (St. Louis, MO, USA). All reagents and solvents used were of analytical grade.

2.2. Animals

All procedures using animals were performed in accordance with the Guidelines on the Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare and approved by the Research Ethics Board of the Metropolitan Autonomous University-Iztapalapa. Adult male and female Wistar rats (body weight: 200–250 g) used throughout the study were kept under a 14 h-light/10 h-darkness cycle and allowed food and water ad libitum. Gonadectomies were performed under light ether anaesthesia. Animals were killed by decapitation and the appropriate tissues were immediately removed, blotted and weighed and then submitted to experimental procedures.

2.3. Incubations

Anterior pituitaries (2 mg protein) and hypothalami (4 mg protein) from female rats ovariectomised 21 days prior to experiments were homogenised in a glass homogeniser

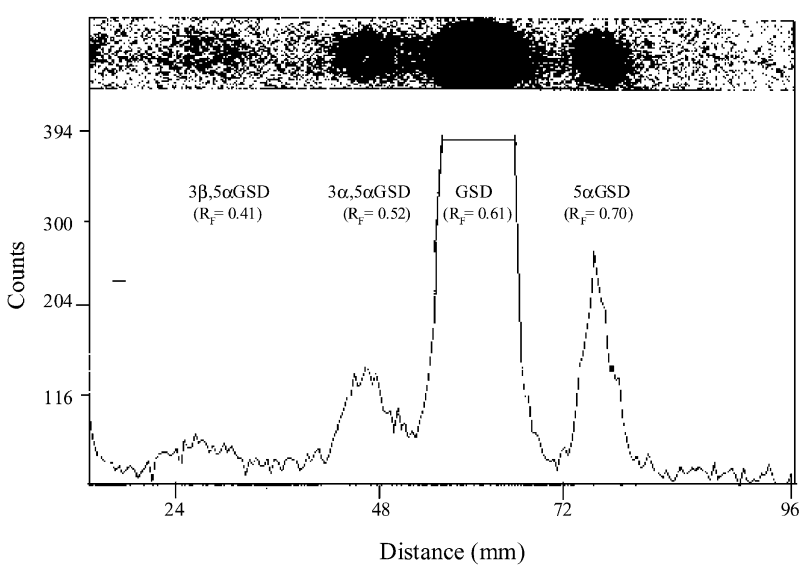

Fig. 1. Representative chromatographic profile of the methanolic extracts of homogenates of rat anterior pituitaries and hypothalami incubated with [^{14}C]-gestodene at pH 7.4. An aliquot was chromatographed on thin-layer plates using two solvent systems. Four radioactive zones were detected, which were identified as 5α-dihydrogestodene (5αGSD), unchanged gestodene (GSD), 3α,5α-tetrahydrogestodene (3α,5αGSD) and 3β,5α-tetrahydrogestodene (3β,5αGSD). Identical results were obtained when [^{3}H]-gestodene was used as substrate. Gestodene was not bioconverted to A-ring reduced derivatives when incubations were done at pH 4.8. For details see the text.

Table 1
Radiochemical purity of metabolites isolated after in vitro incubation of homogenates of rat anterior pituitary, hypothalamus and ventral prostate with [^{3}H]- or [^{14}C]-labelled gestodene (GSD)

Isolated metabolites	Successive crystallisations	Pituitary (dpm[^{3}H]/mg)	Hypothalamus (dpm[^{3}H]/mg)	Prostate (dpm[^{14}C]/mg)
5αGSD	C_1[a]	1424	696	1217
	C_2	1252	524	1186
	C_3	1073	434	1140
	ML_3[b]	1101	487	1164
3α,5αGSD	C_1	1069	1208	517
	C_2	1001	1133	381
	C_3	931	980	327
	ML_3	886	1020	358
3β,5αGSD	C_1	660	523	274
	C_2	492	417	176
	C_3	442	344	147
	ML_3	472	413	154

[a] Crystals.
[b] Mother liquors.

with a Teflon pestle using Krebs–Ringer phosphate buffer solution, in a tissue/buffer ratio of 1:6 (w/v). Rat ventral prostates (8 mg protein) from male rats castrated 48 h before were homogenised using a Polytron homogeniser (Brinkmann Instruments, Westbury, NY, USA). To assess whether gestodene serves as a substrate for both 5α-steroid reductases type 1 and type 2 (Russell and Wilson, 1994), tissues were homogenised using Krebs–Ringer phosphate buffer at two different pHs, 4.8 and 7.4. All procedures were performed at 4°C. Tissue protein was determined by the Bradford dye binding method (Bradford, 1976), using bovine serum albumin as a standard. Tissue preparations were incubated in triplicate with various concentrations (1, 2 and 4 μM) of either [^{3}H]- or [^{14}C]-labelled gestodene in the absence or presence of different concentrations of NADPH (0.5, 1 and 2 mM) in a Dubnoff metabolic incubator at 37.5°C with air as the gas phase for 15, 30 or 60 min. The final incubation volume was 1.0 ml. Incubation of rat anterior pituitary, hypothalamic and ventral prostate homogenates with [^{3}H]-testosterone, under identical experimental conditions, were used as positive controls. Tissue-free incubations served as negative controls.

At the end of the incubation period, the reaction was stopped by addition of ethyl acetate and radiolabelled steroids were extracted (4×) using three volumes of water-saturated ethyl acetate. The organic extracts were partitioned between petroleum ether and 10% aqueous methanol and 2.5 μg each of the steroid carriers gestodene, 5α gestodene, 3α,5α gestodene and 3β,5α gestodene was added to the methanolic extracts. The identification and radiochemical purity of gestodene metabolites were established by a behaviour identical to that of the steroid carriers in two different thin-layer chromatographic systems (chloroform:acetone = 9:1 and benzene:ethyl acetate = 2:1) and recrystallisation to obtain a constant specific activity. [^{3}H]-labelled metabolites were located on chromatographic plates using a Packard radiochromatogram scanner, while [^{14}C]-labelled gestodene derivatives were detected with the use of a Packard instant imager (Downers Grove, IL, USA). Radioactivity was otherwise determined in a Packard Tri-Carb liquid scintillation spectrometer, model 1900 TR using toluene containing 4.0 g/l PPO (2,5-diphenyl-oxazole) and 100 mg/l dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyl-oxazolyl) benzene] as the

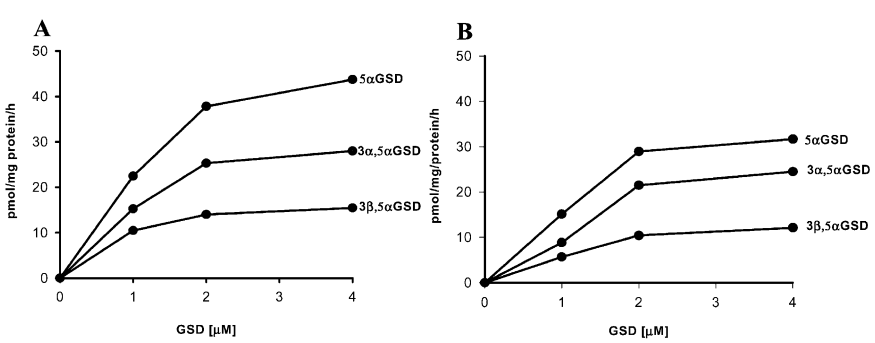

Fig. 2. In vitro metabolism of [^{14}C]-or [^{3}H]-labelled gestodene (GSD) in homogenates of female rat anterior pituitaries (panel A) and hypothalami (panel B) as a function of substrate concentration. 5α-Dihydrogestodene (5αGSD) was identified as the major metabolic conversion product, with 3α,5α-tetrahydrogestodene (3α,5αGSD) and its 3β isomer (3β,5αGSD) being formed in smaller amounts. Optimal gestodene bioconversion to its A-ring reduced metabolites occurred at 2 μM gestodene.

counting solution. Counting efficiencies for [^{14}C] and [^{3}H] were 86% and 67%, respectively, and quenching was corrected in all samples by external standardisation. Non-radioactive steroid carriers were detected on chromatograms using the *p*-anisaldehyde-sulphuric/acetic acids reagent.

Enzyme activities were studied for three gestodene concentrations (1, 2 and 4 μM). The rates of formation of radioactive gestodene and testosterone derivatives are expressed as pmol/mg protein/h. Apparent K_m and V_{max} values of 5α-steroid reductases were determined using an iterative program (Microcal, Origin™).

3. Results

3.1. Incubation of rat anterior pituitary and hypothalamus homogenates with radiolabelled gestodene

After partition of the extracts of rat anterior pituitary and hypothalamus homogenates, 95% of the radioactive material was recovered in the methanolic fraction. When aliquots of methanolic extracts from incubations at pH 7.4 were submitted to thin-layer chromatography, four radioactive zones were detected, as shown in Fig. 1. The less polar zone ($R_F = 0.70$), representing the major metabolic conversion product of both [^{3}H]-gestodene and [^{14}C]-gestodene, had a chromatographic behaviour identical to that of the 5α gestodene carrier. After elution, a representative aliquot was mixed with additional radioinert 5α gestodene and recrystallised to constant specific activity, as shown in Table 1. Zone 2 ($R_F = 0.61$) was identified as unchanged gestodene (Fig. 1), while zones 3 ($R_F = 0.52$) and 4 ($R_F = 0.41$) corresponded to the 3α,5α- and 3β,5α-tetrahydrogestodene metabolites, respectively. Radiochemical purity data of both gestodene tetrahydro derivatives are shown in Table 1. In contrast, chromatographic analysis of the methanolic extracts of homogenates of rat anterior pituitaries and hypothalami incubated at pH 4.8 revealed that gestodene was not bioconverted to A-ring reduced derivatives (data not shown).

Table 2
Rate of formation of A-ring reduced metabolites of [^{3}H]- or [^{14}C]-labelled gestodene (GSD) in rat hormone-sensitive tissues

Incubated tissues	Incubation pHs	5αGSD	3α,5αGSD	3β,5αGSD	DHT[a]
		pmol/mg protein/h			
Anterior pituitary	7.4	37.7	25.3	14.0	165.0
	4.8	ND[b]	ND	ND	ND
Hypothalamus	7.4	29.0	21.5	10.5	111.0
	4.8	ND	ND	ND	ND
Ventral prostate	7.4	55.0	16.3	13.5	222.0
	4.8	69.0	19.8	17.8	318.0

[a]Rates of formation of 5α-dihydrotestosterone (DHT) were determined from incubations with [^{3}H] testosterone used as an experimental control.
[b]ND = Non-detected.

When the metabolism of radiolabelled gestodene in the anterior pituitary homogenate of castrated rats was studied as a function of substrate concentration, it was found that 5α gestodene and 3α,5α gestodene were the major metabolites formed at all substrate concentrations used (Fig. 2, panel A), while the bioconversion of gestodene to 3β,5α gestodene occurred to a significantly lesser extent, as indicated in Fig. 2, panel A. An optimal rate of conversion of gestodene to its reduced derivatives occurred at 2 μM. Results for the rat hypothalamus homogenate incubated with radiolabelled gestodene demonstrated that the formation of its three A-ring reduced metabolites followed the same pattern as that observed in the rat anterior pituitary homogenates, though to a lesser extent, as shown in Fig. 2, panel B. Subsequent experiments were done using [^{3}H]- or [^{14}C]-labelled gestodene at 2 μM. When the metabolism in vitro of radiolabelled gestodene was studied as a function of time, it was found that 1 h was the optimal incubation period in terms of formation of the A-ring reduced metabolites (data not shown). Biotransformation of radiolabelled gestodene to 5α gestodene was NADPH-dependent, as no metabolism was seen in the absence of

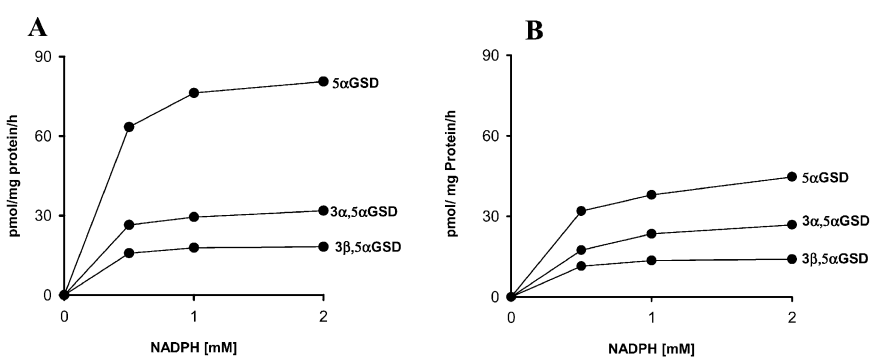

Fig. 3. NADPH-dependence of the in vitro A-ring reduction of [^{14}C]-or [^{3}H]-labelled gestodene (GSD) in rat anterior pituitaries (panel A) and hypothalami (panel B). Bioconversion of gestodene to 5α-dihydrogestodene (5αGSD) was completely dependent on NADPH as no metabolism was noticed in the absence of the cofactor. Optimal concentration of NADPH in both tissues was 1.0 mM.

Table 3
5α-Reductase activity in rat hormone-sensitive tissues measured using radiolabelled gestodene (GSD) or testosterone (T) as substrate in incubations at pH 4.8 and 7.4

Incubated tissues	Incubation at pH 7.4 (5α-reductase type 1)				Incubation at pH 4.8 (5α-reductase type 2)			
	K_m [nM] Substrate		V_{max} [pmol/mg protein/h] Substrate		K_m [nM] Substrate		V_{max} [pmol/mg protein/h] Substrate	
	GSD	T	GSD	T	GSD	T	GSD	T
Anterior pituitary	2.16	0.78	72.46	217.39	ND[a]	ND	ND	ND
Hypothalamus	3.06	1.50	63.29	188.67	ND	ND	ND	ND
Ventral prostate	0.59	0.65	70.86	289.3	0.58	0.65	88.62	387.1

[a] ND = Non-detected.

the cofactor (Fig. 3, panels A and B). The optimal rate of conversion of gestodene to 5α gestodene in both anterior pituitary (Fig. 3, panel A) and hypothalamus (Fig. 3, panel B) homogenates was observed at 1.0 mM NADPH.

The rates of formation of 5α gestodene, 3α,5α gestodene and 3β,5α gestodene in rat anterior pituitary and hypothalamus homogenates incubated at pH 7.4 are depicted in Table 2. Values of apparent K_m and V_{max} for the bioconversion of gestodene to 5α gestodene were determined graphically by Lineweaver–Burk plots and are shown in Table 3. Results of the in vitro 5α-reduction of [^{3}H]-testosterone in rat pituitary and hypothalamus homogenates used as an experimental control are shown in Tables 2 and 3.

3.2. Incubations of rat ventral prostates with radiolabelled gestodene

Gestodene was biotransformed to 5α gestodene, 3α,5α gestodene and 3β,5α gestodene in rat ventral prostate homogenates incubated at two different pHs, 7.4 and 4.8 (Fig. 4, panel A). The optimal substrate concentration at both pHs for the conversion of gestodene to 5α gestodene, 3α,5α gestodene and 3β,5α gestodene was 2 μM and it

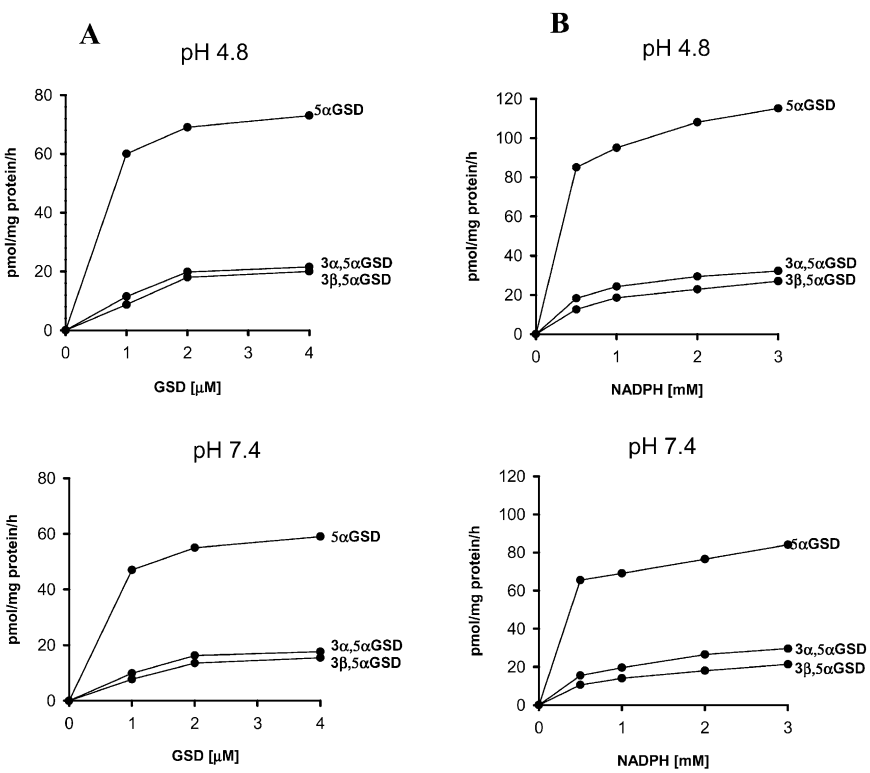

Fig. 4. 5α-Reduction of [^{3}H]- or [^{14}C]-labelled gestodene (GSD) in homogenates of rat ventral prostate as a function of substrate (panel A) and NADPH (panel B) concentrations. Bioconversion of gestodene to 5α-dihydrogestodene (5αGSD) occurred at pH 4.8 and 7.4, with the highest rate of formation at the acidic pH. Optimal bioconversion occurred at 2 μM gestodene. 5α-Reduction of gestodene was completely NADPH-dependent at both pHs with an optimal 1.0–2.0 mM concentration of the cofactor.

was NADPH-dependent (Fig. 4, panel B) in a manner similar to that observed in rat anterior pituitary and hypothalamus homogenates incubated at pH 7.4. The rates of formation of A-ring reduced metabolites of gestodene and testosterone in the rat ventral prostate are shown in Table 2. Values of apparent K_m and V_{max} for rat prostate 5α-reduction of labelled gestodene and testosterone are shown in Table 3. Only unmodified radiolabelled gestodene was recovered from control tissue-free incubations.

4. Discussion

The results of this study provide evidence that gestodene, the most potent synthetic progestin currently available, is extensively metabolised to A-ring reduced derivatives in steroid hormone-sensitive tissues. Indeed, in vitro incubation of [^{3}H]-gestodene and [^{14}C]-gestodene with homogenates of rat anterior pituitary, hypothalamus and ventral prostate resulted in the formation of radioactive 5α gestodene, 3α,5α gestodene and 3β,5α gestodene, thus indicating that gestodene is a substrate for 5α-steroid reductases and 3β- and 3α-hydroxysteroid dehydrogenases in these target organs (Fig. 5). The 5α-dihydro derivative of gestodene was the major metabolic conversion product, whereas the 3β,5α- and 3α,5α-tetrahydro derivatives were formed in smaller amounts, as shown in Table 2 and Fig. 3.

The enzyme-mediated 5α-reduction (Trans A/B ring junction) of gestodene was similar to that of naturally occurring testosterone used as a control (Table 3). The bioconversion of gestodene to 5α gestodene in the rat anterior pituitary and hypothalamus homogenates occurred only at pH 7.4, in an identical manner to that observed when testosterone was used as substrate. These data demonstrate that 5α-steroid reductase (E.C.I. 3.99.5) type 1 reduces the 4–5 double bond of gestodene in these neuroendocrine organs and are in line with a number of reports (Russell and Wilson, 1994; Mahendroo et al., 1996; Celotti et al., 1997), indicating that the type 1 isozyme is widely expressed in the mammalian brain. The lack of biotransformation of gestodene to 5α gestodene at pH 4.8 strongly suggests the absence of the type 2 isoform of 5α-steroid reductase in the anterior pituitary and hypothalamus, confirming and extending previous observations (Normington and Russell, 1992; Thigpen et al.,1993).

In contrast, 5α gestodene was isolated as the major metabolite of gestodene from homogenates of rat ventral prostate incubated at both pH 4.8 and 7.4, thus indicating that A-ring reduction of this synthetic progestin may be mediated by both 5α-steroid reductases type 1 and type 2 (Tables 2 and 3). The presence of the type 2 isozyme in this accessory sex organ has been well documented (Normington and Russell, 1992; Russell and Wilson, 1994; Poletti et al., 1998) and supports the concept that it is selectively expressed in classical male androgen-dependent structures. The enzyme mediated 5α-reduction of gestodene was NADPH-dependent in all tissues studied (Figs. 3 and 4, panel B), as has been extensively reported for testosterone (Massa et al., 1972).

The most remarkable finding of this study was the isolation and identification of 3α,5α gestodene and 3β,5α gestodene as conversion products of gestodene in three target organs, presumably with 5α gestodene as an inter-

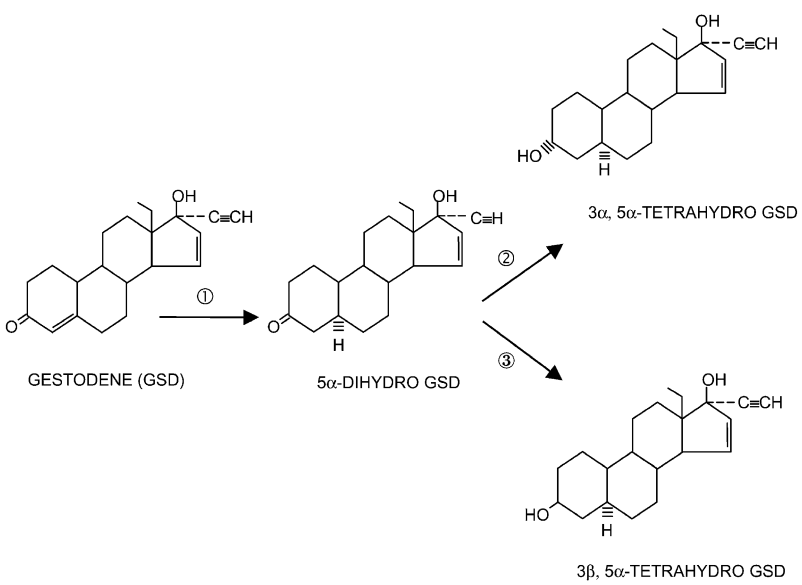

Fig. 5. Metabolic pathways of gestodene (GSD) in hormone-sensitive tissues, as demonstrated in this study by in vitro incubation of [^{3}H]- and [^{14}C]-labelled GSD with homogenates of rat hypothalamus, anterior pituitary and ventral prostate. Radiochemically pure 5α-dihydrogestodene, 3α,5α-tetrahydrogestodene and 3β,5α-tetrahydrogestodene were isolated as metabolic conversion products, indicating that gestodene is an adequate substrate for the 5α-steroid reductases type 1 and type 2 (①), 3α-hydroxysteroid dehydrogenase (②) and 3β-hydroxysteroid dehydrogenase (③).

mediate. Although the pharmacokinetics of gestodene after its oral and intravenous administration have been studied (Täuber et al., 1989) and some A-ring reduced gestodene metabolites have been partially identified from human liver incubations (Ward and Back, 1993), this is the first report of the characterisation of neutral reduced gestodene metabolites in hormone sensitive-organs.

Taken together with the recent observations that 3β,5α and 3α,5α gestodene are able to activate co-transfected yeast and HeLa cells cultures with vectors containing the human oestrogen receptor α and oestrogen-dependent reporter genes (Lemus et al., 2000), these results seem to indicate that the slight oestrogenic effects of gestodene are most likely mediated by its tetrahydro metabolites. Further support for this concept is furnished by the demonstration that the 3β,5α derivative of gestodene is able to induce in vivo oestrogen-dependent progesterone receptors in the castrated female rat, yet with a significantly lower potency than that of oestradiol benzoate (Lemus et al., 2000). The finding that gestodene has oestrogen agonistic effects, exerted through its enzyme-mediated formed metabolites, is in line with previous reports indicating that norethisterone, another 19-nor contraceptive progestin, induces oestrogenic actions through its non-phenolic reduced metabolites (Pérez-Palacios et al., 1993; Oropeza et al., 1994). Overall, the data contribute to a better understanding of the mode of action of gestodene and underline the role of peripheral metabolism of synthetic progestins in the modulation of their hormone-like effects.

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References

Bardin, C.W., 1983. The androgenic, antiandrogenic and synandrogenic actions of progestins. In: Bardin, C.W., Milgröm, E., Mauvais-Jarvis, P. (Eds.), Progesterone and Progestins. Raven Press, New York, NY, pp. 135–147.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Castro, I., Cerbón, M.A., Pasapera, A.M., Gutiérrez-Sagal, R., García, G.A., Orozco, C., Camacho-Arroyo, I., Anzaldua, R., Pérez-Palacios, G., 1995. Molecular mechanisms of the antihormonal and antiimplantation effects of norethisterone and its A-ring reduced metabolites. Mol. Reprod. Dev. 40, 157–163.

Catherino, W.H., Jeng, M.H., Jordan, V.C., 1993. Norgestrel and gestodene stimulate breast cancer cell growth through an oestrogen receptor mediated mechanism. Br. J. Cancer 67, 945–952.

Celotti, F., Negri-Cesi, P., Poletti, A., 1997. Steroid metabolism in the mammalian brain: 5 alpha-reduction and aromatization. Brain Res. Bull. 44, 365–375.

Chávez, B.A., Vilchis, F., Pérez, A.E., García, G.A., Grillasca, I., Pérez-Palacios, G., 1985. Stereospecificity of the intracellular binding of norethisterone and its A-ring reduced metabolites. J. Steroid Biochem. 22, 121–126.

Düsterberg, B., Tack, J.W., Jrause, W., Humpel, M., 1987. Pharmacokinetics and biotransformation of gestodene in man. In: Elstein, M. (Ed.), Gestodene. Development of a New Gestodene-containing Low Dose Oral Contraceptive. Parthenon Publishing Group, Carnforth, pp. 35–44.

Jick, H., Jick, S.S., Gurewich, V., Myers, M.W., Vasilakis, C., 1995. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. Lancet 346, 1589–1593.

Kalkhoven, E., Kwakkenbos-Isbrüker, L., de Laat, S.W., Van der Saag, P.T., Van der Burg, B., 1994. Synthetic progestins induce proliferation of breast tumor cell lines via the progesterone or estrogen receptor. Mol. Cell. Endocrinol. 102, 45–52.

Lemus, A.E., Vilchis, F., Damsky, R., Chávez, B.A., García, G.A., Grillasca, I., Pérez-Palacios, G., 1992. Mechanism of action of levonorgestrel: in vitro metabolism and specific interactions with steroid receptors in target organs. J. Steroid Biochem. Mol. Biol. 41, 881–890.

Lemus, A.E., Zaga, V., Santillán, R., García, G.A., Grillasca, I., Damián-Matsumura, P., Jackson, K.J., Cooney, A.J., Larrea, F., Pérez-Palacios, G., 2000. The oestrogenic effects of gestodene, a potent contraceptive progestin, are mediated by its A-ring reduced metabolites. J. Endocrinol. 165, 693–702.

Lewis, M.A., Heinemann, L.A.J., MacRae, K.D., Bruppacher, R., Spitzer, W.O., 1996. The increased risk of venous thromboembolism and the use of third generation progestagens: role of bias in observational research. Contraception 54, 5–13.

Mahendroo, M.S., Cala, K.M., Russell, D.W., 1996. 5α-reduced androgens play a key role in murine parturition. Mol. Endocrinol. 10, 380–392.

Massa, R., Stupnicka, E., Kniewald, Z., Martini, L., 1972. The transformation of testosterone into dihydrotestosterone by the brain and the anterior pituitary. J. Steroid Biochem. 3, 385.

Moralí, G., Lemus, A.E., Oropeza, M.V., García, G.A., Pérez-Palacios, G., 1990. Induction of male sexual behavior by norethisterone: Role of its A-ring reduced metabolites. Pharmacol. Biochem. Behav. 37, 477–484.

Mowszowicz, I., Bieber, D.E., Chung, K.W., Bullock, L.P., Bardin, C.W., 1974. Synandrogenic and antiandrogenic effect of progestins: comparison with non-progestational antiandrogens. Endocrinology 95, 1589–1599.

Normington, K., Russell, D.W., 1992. Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozymes. J. Biol. Chem. 267, 19548–19554.

Oropeza, M.V., Campos, M.G., Lemus, A.E., García, G.A., Pérez-Palacios, G., Ponce-Monter, H., 1994. Estrogenic actions of norethisterone and its A-ring reduced metabolites. Induction of in vitro uterine sensitivity to serotonin. Arch. Med. Res. 25, 307–310.

Pasapera, A.M., Cerbón, M.A., Castro, I., Gutiérrez, R., Camacho-Arroyo, I., García, G.A., Pérez-Palacios, G., 1995. Norethisterone metabolites modulate the uteroglobin and progesterone receptor gene expression in prepubertal rabbits. Biol. Reprod. 52, 426–432.

Pérez-Palacios, G., Chávez, B., Vilchis, F., Escobar, N., Larrea, F., Pérez, A.E., 1983. Interaction of medroxyprogesterone acetate with cytosol androgen receptors in the rat hypothalamus and pituitary. J. Steroid Biochem. 19, 1729–1735.

Pérez-Palacios, G., Moralí, G., García, G.A., Cruz, M.L., Lemus, A.E.,

1993. Behavioral effects of synthetic progestins. In: Mornex, R., Jaffiol, C., Leclere, J. (Eds.), Progress in Endocrinology. Parthenon Publishing Group, London, New York, pp. 155–159.

Poletti, A., Negri-Cesi, P., Rabuffetti, M., Colciago, A., Celotti, F., Martini, L., 1998. Transient expression of the 5α-reductase type 2 isozyme in the rat brain in late fetal and early postnatal life. Endocrinology 139, 2171–2178.

Russell, D.W., Wilson, J.D., 1994. Steroid 5α-reductase: two genes/two enzymes. Annu. Rev. Biochem. 64, 25–61.

Schoonen, W.G.E.J., Joosten, J.W.H., Kloosterboer, H.J., 1995. Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: I. MCF-7 cell lines. J. Steroid Biochem. Mol. Biol. 55, 423–437.

Täuber, U., Tack, J.W., Matthes, H., 1989. Single dose phamacokinetics of gestodene in women after intravenous and oral adminstration. Contraception 40, 461–479.

Thigpen, A.E., Cala, K.M., Russell, D.W., 1993. Characterization of Chinese hamster ovary cell lines expressing human steroid 5α-reductase isozymes. J. Biol. Chem. 268, 17404–17412.

Van der Burg, B., Kalkhoven, E., Isbrücker, L., de Laat, S.W., 1992. Effects of progestins on the proliferation of estrogen-dependent human breast tumor cells under growth factor-defined conditions. J. Steroid Biochem. Mol. Biol. 42, 457–465.

Vilchis, F., Chávez, B., Pérez, A.E., García, G., Angeles, A., Pérez-Palacios, G., 1986. Evidence that a non-aromatizable metabolite of norethisterone induces estrogen-dependent pituitary progestin receptors. J. Steroid Biochem. 24, 525–531.

Ward, S., Back, D.J., 1993. Metabolism of gestodene in human liver cytosol and microsomes in vitro. J. Steroid Biochem. Mol. Biol. 46, 235–243.

World Health Organization (collaborative study of cardiovascular disease and steroid hormone contraception), 1995a. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case-control study. Lancet 346, 1575–1582.

World Health Organization (collaborative study of cardiovascular disease and steroid hormone contraception), 1995b. Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. Lancet 346, 1582–1588.